

Histamine release from basophils in cystic fibrosis

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SUMMARY

We determined the histamine release from basophils in patients suffering from cystic fibrosis (CF) (median 17.2 years of age) and compared the data with an age-matched group of healthy donors. No significant differences in the basophil counts were determined between the CF and control groups. However, the absolute histamine content per basophil was elevated in the CF group (2.6 ± 0.4 pg/basophil versus 1.4 ± 0.2 , mean \pm s.e.m., $n = 15/10$, $P < 0.004$). Stimulation of basophils with the Ca ionophore ($7.5 \mu\text{M}$) and anti-IgE (10^{-2} of a stock preparation) leads to a significantly higher release of histamine per basophil in CF patients as compared to healthy donors (Ca ionophore: 1.6 ± 0.2 versus 0.9 ± 0.2 , mean \pm s.e.m., $P < 0.008$; anti-IgE: 0.45 ± 0.007 versus 0.28 ± 0.04 , $P < 0.02$). These data indicate that basophils in CF may have a greater potential to release mediators although their releasability, expressed as the percentage of histamine release of the total histamine content, does not differ significantly compared to the healthy donors ($62.1 \pm 8.3\%$ versus $60.2 \pm 13\%$, mean \pm s.e.m.). Within the 14-day period of intravenous antibiotic treatment of the pulmonary infection (in 14 out of 15 cases *P. aeruginosa* was isolated from sputa samples) histamine release per basophil and total histamine content decreased to normal levels (day 1 2.6 ± 0.3 versus 1.8 ± 0.3 , $P < 0.05$). This decline was accompanied by an improvement of the clinical condition of the patient and reduction of *P. aeruginosa* isolates in sputa ($n = 8$). In contrast, in three patients with sustained *P. aeruginosa* colonization of the upper airways and impaired lung function histamine levels remained elevated. Our data demonstrate that the histamine content of basophils, as well as the release of histamine, is increased in patients with cystic fibrosis and correlates with the clinical signs of the chronic infection.

Keywords cystic fibrosis basophils histamine release

INTRODUCTION

Cystic fibrosis (CF) is the most common genetically determined metabolic disorder in children and adolescents (Wood, Boat & Doershuk, 1976). A constant finding is the defect in the excretory and eccrine glands which leads to the production of an abnormal viscous, dehydrated mucus with malfunction and destruction of the exocrine pancreas and the lung leading to a severe chronic obstruction and infection with an impaired mucociliary clearance. As is the case for immune compromised patients, such as burned, chronic diseased and hospitalized patients, infection with the opportunistic pathogen *P. aeruginosa* is regularly observed in cystic fibrosis (Høiby, 1982). Several humoral, as well as cellular, immunological alterations are described in CF, such as elevated levels for immunoglobulins (IgG, especially IgG4, IgM and IgA); in the bronchoalveolar space the release and generation of pro-inflammatory sub-

stances such as lipid mediators e.g. cysteinyl leukotrienes (Zakrzewski *et al.*, 1987) and granular enzymes (e.g. elastase) (McFarlane, 1986; Wilson, 1986).

Over recent years it has become clear that basophils and mast cells are not only involved in allergic reactions but play a crucial role in inflammatory processes. Basophils are known to be attracted into the inflamed tissue and can be stained on surface areas of the upper airways (Hastie *et al.*, 1979). Their capacity to generate potent mediators such as arachidonic acid metabolites (e.g. LTC₄) and the release of preformed mediators, such as histamine and heparin, influence the local inflammatory process. Generation and release of these substances is observed in different cells (e.g. granulocytes, macrophages, basophils, mast cells) upon stimulation with bacterial toxins and bacteria themselves (König *et al.*, 1986). Histamine causes edema formation, contraction of smooth muscle cells, activation of other inflammatory cells, and it regulates local immunity by its ability to influence lymphocyte function (Owen, 1987).

Only few data exist about the role of immediate-type hypersensitivity mediators such as histamine in cystic fibrosis. Therefore, it was the purpose of our study to investigate

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histamine release from basophils in patients suffering from cystic fibrosis and to correlate it with clinical conditions and immunological parameters during anti-infectious therapy.

MATERIALS AND METHODS

Patients

Two groups of patients were compared. In Group I (CF) the diagnosis of CF was verified by repeated sweat test according to Gibson & Cooke (1959). Fifteen patients aged between 8 and 22 years (median: 17.2, 14.8 ± 5.3 years, mean \pm s.d.) were studied. In all but two patients an exacerbation of the inflammatory process in the lung was diagnosed, and this led to the hospitalization for intravenous antibacterial therapy.

Clinical status. Clinical examination was performed before entrance into the study and enclosed X-ray of the chest (classified according to Chrispin and Norman, 1974), the clinical score (according to Kraemer *et al.*, 1978), laboratory data and lung function (see Table 1). Lung function was evaluated by spirometry (Pneumoscreen, Jaeger, Würzburg, FRG). Immunoglobulin levels were determined either by radial immunodiffusion (NOR-Partigen plates for IgM, IgG and IgA, Behring AG, Marburg FRG), or by an IgE-specific enzyme-linked immunoassay (Enzygnost IgE-monoklonal, Behring AG, Marburg FRG). The Ig-levels were determined as s.d.s. (standard deviation score):

$$\frac{\text{actual value} - \text{mean of the age-matched control group}}{\text{standard deviation}}$$

The SDS covers the age-dependency of Ig-levels in comparison to the levels of healthy donors (Cejka, Mood & Kim 1974; Kjellman, Johansson & Roth 1976); by definition normal levels of the s.d.s. range between 0 ± 1 .

Microbiology. In 14 out of 15 cases *P. aeruginosa* strains were isolated from the sputa. Up to five different strains could be identified in individual patients, mucoid variants were identified in all patients. In addition, *A. fumigatus* (1/15), *C. albicans* (2/15) and β -haemolytic *Streptococci* species (1/15) were isolated.

Therapy. Intravenous antibacterial therapy was performed over a period of 14 days in 11/15 cases. The therapeutic regimen was based on the resistograms of the strains; predominantly azlocillin (150 mg/kg/day) in combination with tobramycin (10 mg/kg/day) were used, if necessary replaced by piperacillin, ceftazidime or ciprofloxacin. This therapy was supported by chest physiotherapy and inhalation of sodium chloride combined with antibiotics (tobramycin 80 mg) and mucolytics and bronchodilators (acetylcysteine, salbutamolsulphate).

Group II comprised 10 healthy children (8–15 years of age, median 11 years, 12 ± 2.6 mean \pm s.d.) studied in parallel with the CF group. Acute or chronic inflammatory processes, allergic diathesis and immune disorders were excluded by patients history and clinical examination. Laboratory findings such as WBC and differential count, ESR, haemoglobin, immunoglobulin concentrations and C-reactive protein were in the normal range.

Materials

All fine chemicals were purchased from Merck Darmstadt, FRG and Baker, Deventer, Holland. Ca-ionophore was purchased from Sigma, Munich, FRG. Anti-human-IgE antibody (Tago Inc., Burlingame CA, USA), which was purified by

affinity chromatography, was used in dilutions of 10^{-2} to 10^{-4} (stock solution 0.25 mg/ml antibody). The antibody showed a cross-reactivity against IgG < 2.5%. Phosphate-buffered saline (PBS) was used for cell isolation and stimulation experiments.

Isolation of basophils

Peripheral blood was collected on day 1 before therapy, and on days 3, 7 and 16 after therapy in the CF group.

Isolation of blood cells

Heparinized blood. Twenty-five millilitres were overlaid on a Ficoll metrizoate gradient as described elsewhere (König *et al.*, 1986). After centrifugation (40 min, at 400 g, 4°C) the lymphocyte, monocyte and basophil containing fraction (LMB-fraction) was carefully removed and washed twice in PBS buffer. The basophil content was determined after alcian blue staining according to Gilbert & Ornstein (1975).

Incubation procedure. The cells were diluted up to a final concentration of 2×10^6 cells/500 μ l in PBS-buffer. The incubation (30 min at 37°C) was carried out in the presence of Ca/Mg (1/0.5 mM). The Ca ionophore (final concentration 7.3 μ M) and 50 μ l anti-IgE solution (10^{-2} , 10^{-3} and 10^{-4} of the stock solution) served as stimulus. Spontaneous histamine release in the presence of Ca/Mg in the absence of any stimulus served as a control.

Table 1. Clinical and laboratory parameters of the patients under study

Parameter	Mean \pm s.e.m.	Normal levels
pO ₂	68.7 \pm 3.4*	74–108 Torr
pCO ₂	38.4 \pm 1.8	2.5–46.6 Torr
FEV ₁	41.5 \pm 6.7*	100%
FVC	53.6 \pm 4.9*	100%
Body weight	−4.1 \pm 1.9*	0
Clinical score	18 \pm 1.0*	25
X-ray score	16 \pm 2.1*	0
IgG SDS	3.9 \pm 1.0*	1 < 0 < −1
IgM SDS	1.8 \pm 0.36*	1 < 0 < −1
IgA SDS	2.7 \pm 0.51*	1 < 0 < −1
IgE SDS	6.0 \pm 3.3*	1 < 0 < −1
Anti-exotoxin A	> 1:10	> 1:10
Anti-elastase	1:44 \pm 12.8*	> 1:10
Anti-alk. protease	1:58 \pm 11.6*	> 1:10
CRP	1.9 \pm 0.83*	< 0.8 mg%
ESR (1 h)	38 \pm 0*	3–12 mm/h
C3c	110 \pm 4.6	55–120 mg/dl
C4	38 \pm 5.4	20–50 mg/dl

Ig levels: expressed as SDS; anti-exotoxin A, anti-elastase, anti-alk. protease: antibody titres against the three different *P. aeruginosa* toxins (Döring *et al.*, 1983); clinical score: according to the index of Kraemer *et al.* (1978), normal range 25 points; X-ray score: according to Chrispin & Norman (1974), normal range 0 points; body weight: relative underweight (weight loss corrected for height according the curves of Tanner *et al.* 1966).

CRP C-reactive protein; ESR erythrocyte sedimentation rate.

* Significantly different ($P < 0.05$) as compared to the control group.

Histamine release

The histamine content of the cell supernatants was determined by the fluorophotometrical autoanalyser technique (Siraganian, 1974). The technical equipment was purchased from Technicon (Bad Vilbel, FRG). The histamine concentration was calculated by external standardization; peak area integration was performed with a chromatographic integrator (D-2000, Hitachi Merck, Darmstadt, FRG). Histamine release was calculated as percentage of the total histamine content or as mass (pg) per basophil, as indicated.

Statistical analysis

All data were calculated as mean \pm standard error of the mean (s.e.m.) and the significance was evaluated with Student's *t*-test for independent means; $P < 0.05$ was considered significant.

RESULTS

We studied the histamine release of basophils from patients with cystic fibrosis. The patients suffered from an exacerbation of the chronic inflammatory process in the lung which was caused by infection with *P. aeruginosa*. The mean time of colonization of the upper respiratory tract with *P. aeruginosa* isolates was about 32 ± 6 months (mean \pm s.e.m.) with a minimum of 12 months. All patients had signs of an impaired lung function as can be seen from Table 1. Moreover, the clinical score and the X-ray score indicated a severe regression of the clinical status as compared to the healthy donors. Due to the clinical and cellular signs of chronic infection the patients showed elevated levels of immunoglobulin in serum, especially for the total IgE. In all patients specific antibodies against *P. aeruginosa* were detected, significantly elevated were the levels against the alkaline protease and *P. aeruginosa* elastase. Blood cell counts indicated that in the CF group signs of inflammatory processes such as elevated levels of granulocytes (CF group: 57.1 ± 11.6 control

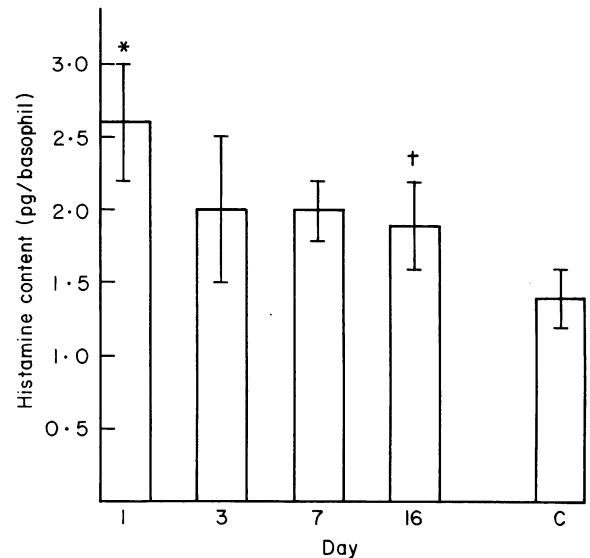


Fig. 1. Histamine content of basophils from health donors and CF patients: Column 1-4: CF patients ($n=11-15$), days 1, 3, 7 and 16 of intravenous antibiotic treatment; column C: control group of healthy donors ($n=10$).

* Significantly elevated as compared to the control group ($P < 0.004$); † significantly decreased as compared to day 1 ($P < 0.05$).

group: $48.8 \pm 7.6\%$) and a shift to immature granulocytes (10.1 ± 2.3 versus $6.9 \pm 1.5\%$) were present, combined with an increase of the total cell number (10.1 ± 2.8 versus $6.9 \pm 1.5 \times 10^3$ cells/mm³). The basophil content (calculated in the lymphocyte, basophil and monocyte containing cell fraction) did not differ significantly between groups (1.0 ± 0.6 versus $0.9 \pm 0.5\%$), as was the case for eosinophils (3.5 ± 1.8 versus 2.5 ± 2.0) and monocytes (8.1 ± 3.7 versus $8.9 \pm 5.7\%$).

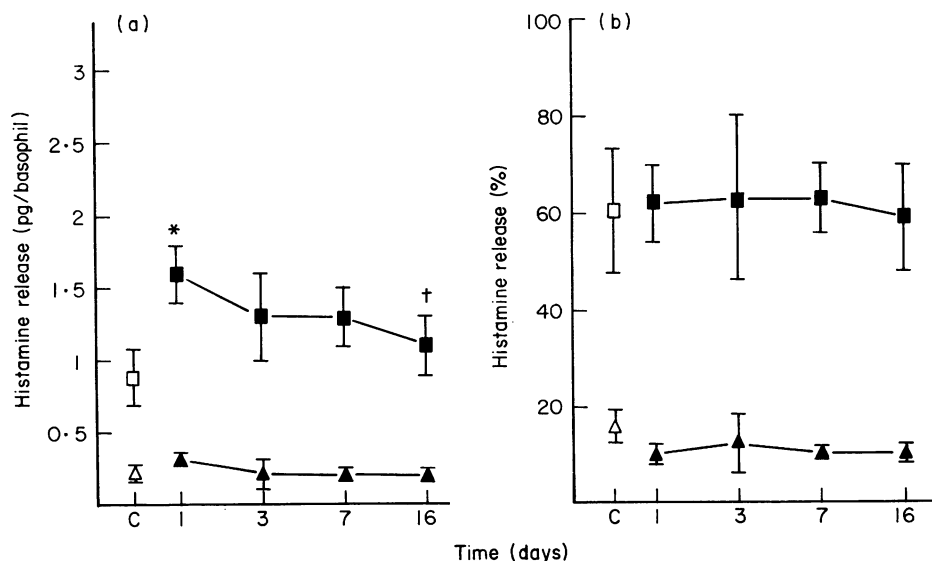


Fig. 2. Histamine release after Ca ionophore stimulation from CF patients (days 1, 3, 7 and 16) and healthy donors (C). Histamine release pg/basophils (a); histamine release of total histamine content (b). Spontaneous histamine release without a secretagogue in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ (\blacktriangle). Histamine release in the presence of Ca ionophore ($7.3 \mu\text{M}$ 30 min, 37°C) (\blacksquare), mean \pm s.e.m.

* Significantly elevated as compared to the control group ($P < 0.008$); † significantly decreased as compared to day 1 ($P < 0.05$).

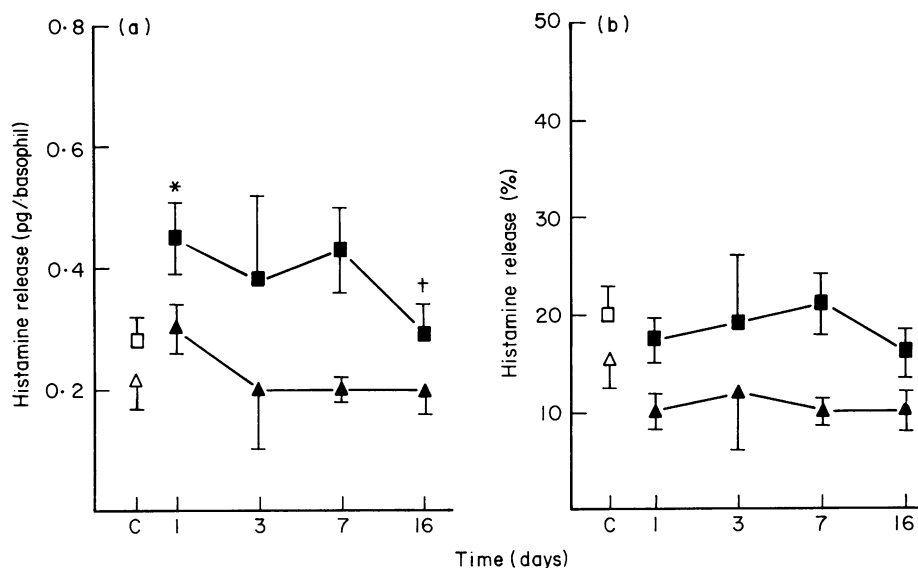


Fig. 3. Histamine release after anti-IgE stimulation (dilution of 10^{-2} of the stock, 30 min, 37°C) from CF patients (days 1, 3, 7 and 16) and healthy donors (C). Histamine release (pg/basophil) (a); Histamine release as percentage of total histamine content (b). Spontaneous histamine release without a secretagogue in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ (\blacktriangle); anti-IgE: Stimulation with anti-IgE at a dilution of 10^{-2} of the stock (\blacksquare), mean \pm s.e.m.

* Significantly elevated as compared to the control group ($P < 0.04$); † significantly decreased as compared to day 1 ($P < 0.05$).

According to the clinical signs and the microbial diagnosis of *P. aeruginosa* strains intravenous antibiotic therapy was performed for 14 days under hospitalized conditions. Stimulation experiments with basophils were carried out before therapy, on days 3, 7 and 16.

Figure 1 summarizes the data of the total histamine content of all CF patients on days 1, 3, 7 and 16, and of healthy donors. On day 1 the histamine content per basophil in the CF group differed significantly from the healthy donors (2.6 ± 0.3 versus 1.4 ± 0.2 , $P < 0.004$). These levels decreased significantly (day 1: 2.6 ± 0.3 day 16: 1.8 ± 0.3 , $P < 0.05$) during the therapy and achieved almost normal levels on day 16 of therapy. No significant difference between healthy donors and CF-patients could be observed on day 16 after therapy.

Table 2. Correlation between clinical and laboratory parameters determined on day 1 of CF patients ($n = 15$) and histamine release

Immunological		Clinical	
Parameter	Corr. coeff.*	Parameter	Corr. coeff.*
Serum levels of			
IgG	-0.21	pO ₂ s	0.10
IgM	-0.16	pCO ₂	0.41
IgA	-0.11	FEV1	0.15
IgE	0.21	FVC	0.19
Complement		X-ray score	-0.24
C3c	-0.003	Clinical score	0.19
C4	-0.31	Relative underweight	0.23
C-reactive protein	-0.12	Number of	
ESR	-0.07	<i>P. aeruginosa</i> isolates	0.07
Leucocyte count	0.26		

* Correlation coefficient of linear regression analysis.

Figure 2 summarizes the data of the stimulation experiments. Incubation with the Ca-ionophore leads to a significantly higher release of histamine per basophil in the patient group (Fig. 2a). This increased release of histamine reflects the elevated histamine content in basophils of CF patients. If one calculates the histamine release as percentage of total histamine content no significant differences between groups could be established (CF: $62 \pm 8\%$ versus $60 \pm 13\%$, Fig. 2b).

A comparable profile was obtained for the anti-IgE stimulation (CF: 0.5 ± 0.1 versus 0.3 ± 0.04 , $P < 0.02$) although the

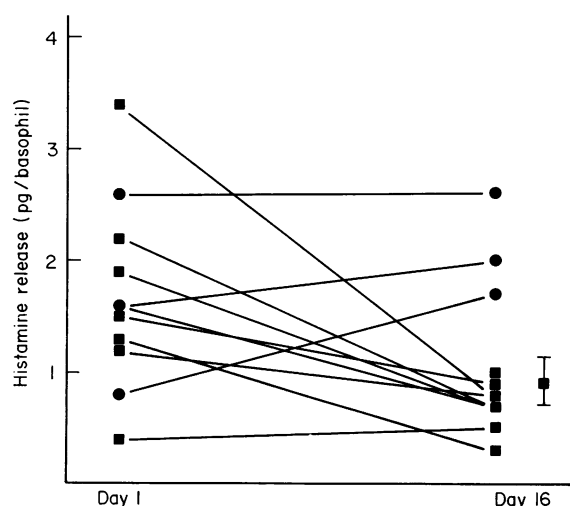


Fig. 4. Individual release of histamine after Ca ionophore stimulation from basophils of CF patients. Day 1: before therapy, day 16: after therapy. Normal range: mean \pm S.D., ■—■: Patients with improved lung function and reduction of *P. aeruginosa* colonization (■); ●—●: patients with sustained reduced lung function and sustained *P. aeruginosa* colonization (●).

histamine release was lower as compared to the Ca-ionophore stimulation. At the highest concentration of anti-IgE used (10^{-2}) a significantly higher release of histamine was observed on day 1 with regard to day 16 (Fig. 3a, b). No significant differences were obtained with anti-IgE stimulation at dilutions of 10^{-3} and 10^{-4} of the stock as compared to the buffer control (data not shown).

Based on different immunological and clinical parameters the correlation coefficient as compared to the histamine release was determined (Table 2). Within the group of patients with cystic fibrosis no correlation was established between several immunological and clinical parameters on day 1 and the corresponding histamine release.

In order to correlate the histamine release with the clinical condition before and after therapy the clinical and immunological parameters which serve as an index of the individual disease process were compared with the individual histamine release on day 1 (before therapy) and day 16 (Fig. 4). In those patients whose clinical status was improved (e.g. significant increase in FEV1), and in whom a significant reduction of *P. aeruginosa* strains isolated from sputa was observed, the histamine release returned to normal levels. However, in three patients (Fig. 4) with sustained elevated levels of histamine the clinical symptoms were not improved and the colonization with *P. aeruginosa* strains could not be reduced.

DISCUSSION

Our data indicate that basophils of CF-patients with an exacerbation of the pulmonary infection due to *P. aeruginosa* infection have a higher content of histamine. The releasability of basophils, defined as the percentage of histamine release of total histamine content, does not differ significantly between healthy donors and CF-patients. However, a higher absolute amount is released after stimulation with the Ca-ionophore as well as with anti-IgE.

In contrast Stahl Skov *et al.* (1980) reported a comparable profile between healthy donors and CF patients. These differences might be due to the different clinical conditions of the patients since the histamine content in their study ranged about the levels which we obtained after the anti-infectious treatment. Moreover, the median age was about 11 years whereas our patients were about 17 years of age. Interestingly, IgE levels of their patients were not elevated.

The question arises whether these observations are due to the primary defect of the cystic fibrosis itself or express secondary inflammatory processes. *P. aeruginosa* and some of its pathogenicity factors, such as production of slime, endotoxins, exotoxin A, proteases and haemolysins (glycolipid, phospholipase C), provokes different effects in the cellular and humoral immune system (Döring & Høiby, 1983; Laharrague *et al.*, 1984). Whether they influence basophil histamine release and histamine content remains to be studied. Although no data are available about the release of histamine from basophils of CF patients immediately after delivery, the data which showed a correlation between the severity of the individual stage of illness and the trend to normalization during the therapy argue against a primary defect of basophils but for a secondary reaction due to the chronic process of bacterial infection of the lung.

Different reasons may contribute to the observed alterations

as compared to healthy donors. The data suggest an altered process of basophil maturation and differentiation. Recently it was described that interleukins (IL), especially IL-3, play an important role with regard to basophil differentiation (Ihle *et al.*, 1983; Smith & Rennick, 1986). It is not known whether the synthesis and biological response of the cytokines, as well as the cellular behaviour of stem cells from which basophils are derived, are altered in cystic fibrosis or in other chronic infectious conditions.

The release of histamine after stimulation predominantly suggests a direct interaction between the stimuli, the Ca ionophore and anti-IgE, with basophils. However, both stimuli also interact with other cells such as monocytes, lymphocytes and platelets, which are present in the basophil-containing cell suspension. It is known that the Ca ionophore and anti-IgE (via low affinity Fc_ϵ receptors) activate these cells (Joseph *et al.*, 1983). In response to these stimuli, histamine releasing factors (HRF), which are described in supernatants of monocytes (Liu *et al.*, 1986), as well as biological fluids such as bronchoalveolar lavage fluid (Gittlen *et al.* 1988), may further influence the histamine release from basophils. Interestingly, the IgE levels are significantly elevated in the CF patients, as also described by others (Rachelefsky *et al.* 1974; Tacier Eugster, Wüthrich & Meyer, 1978). During recent years it has become evident that the regulation of IgE levels is controlled by T cells and depends on an interaction between T and B lymphocytes and other cells. T-cell-derived isotype-specific lymphokines such as IgE-binding factors have been described in experimental animals and in man (Ishizaka, 1988). These factors either enhance or suppress IgE production. Furthermore, interleukins such as IL-4, solely or in combination with other factors, increased the polyclonal IgE synthesis *in vitro* as well as *in vivo* (Paul & Ohara 1987). Interestingly, Plaut *et al.* (1988) observed the release of IL-4 activity from murine mast cells and myeloid cell lines after stimulation with Ca ionophore and IgG immune complexes. Elevated levels of immune complexes are found in the serum of CF patients and it has to be proven whether there is a link between these interleukins, histamine content and release, as well as the levels of IgE. Furthermore, studies are required to compare histamine release with the generation of other mediators. It has to be established that an increased inflammatory potential—as it is suggested by our data for histamine and by the determination of leukotriene C4 levels in the sputum of CF patients—is released into the microenvironment after sufficient stimulation, since the complex network of the inflammatory process *in vivo* includes more than one mediator (Wanner *et al.*, 1987) and depends on the interaction of different cells.

No correlation with several laboratory parameters of inflammation or with the individual clinical status of the patients before therapy and either the histamine release or histamine content of basophils could be established. However, during the treatment with antibiotics a significant improvement of the clinical status of the patients, expressed as an increased FEV1, and a successful antibiotic treatment, expressed in a reduction of *P. aeruginosa* isolates, correlate with the normalization of histamine release per basophil and histamine content of basophils. Other parameters showed no changes during the time period of 16 days due to their slow response to the therapy.

In summary, our results indicate an altered profile of histamine content and release of basophils from CF-patients. Although the reasons for these alterations remain to be

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